Biochemical changes in the midgut during metamorphosis in *Apis cerana indica*

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Abstract

The digestive cells of midgut are responsible for the secretion of various enzymes and absorption of nutrients. During the process of metamorphosis midgut passes through, histolysis and histogenesis. As a result, destruction of larval tissue and construction of adult tissue occurs. The present work thus carried out is to know the changes that occurr in the biomolecules like DNA, RNA, Proteins, Carbohydrates etc., in relation with the remodeling of gut. Along with these biomolecules, various enzymes like amylase, invertase, protease and lipase are also estimated to know their status during metamorphosis of midgut in *Apis cerana indica*.

Keywords- *Apis cerana indica, Midgut, Metamorphosis, Biomolecules*.

Introduction

Apis cerana indica is one of the indigenous species of honeybees, used for apiculture in India. The alimentary canal of the bee can be divided into three distinct regions foregut, midgut and hindgut (Snodgrass, 1935 and 1956). The midgut is the main site for the digestive processes in most of the insects (Wigglesworth, 1972 and House, 1974), the midgut epithelium is composed of columnar cells which secretes the enzymes, carries out digestion and absorption (Wigglesworth, 1977). The ultrastructural and cytochemical aspect indicates that the midgut is remodeled during metamorphosis and shows the programmed cell death in the honey bee larvae and pupae (Gregorc and Bowen, 1996 and 1997, Cruz-landim and Cavalcante 2002 and 2003, Neves *et al.*, 2003 and Barsagade and Kelwadkar, 2008). The peptides of insect brain and midgut are suggested to have important regulatory role in digestive processes like, enzyme secretion, epithelial tissue regeneration, absorption of nutrients, working of gut musculature and maintenance of gut pH (Prabhu and Sreekumar, 1994 and Sunitha *et al.*, 1999). In *Apis cerana indica* remodeling of gut occurs through histolysis and histogenesis during metamorphosis but no information is available regarding biomolecule concentration and protein profile in the midgut cells during metamorphosis. Therefore, present work has been undertaken to know the changes in biomolecules, enzymes and protein profile in the midgut epithelial cells of larvae and pupae of *Apis cerana indica*.

Materials and Methods

Biochemical Techniques

Extract Preparation:

The midguts were dissected out from the

larval and pupal stages, in ice-cold saline/ Ringer's solution. The dissected midguts were homogenized using pestle and mortar for 5 minutes at room temperature in the Ringer's solution. 25 mg tissue/ml volumes of Ringer solution was added, sample was homogenized at 3000 rpm and the supernatant after centrifugation was used for estimation of total concentration of protein according to the Lowery *et al.,* (1951) method. The total carbohydrate was estimated by Phenol-Sulphuric acid method of Dubois *et al.,* (1962), DNA and RNA by Bruton's Diphenylamine and Dische-Orcinol methods of Searcy and MacInnis (1970a and 1970b).

Biochemical Estimation of Digestive Enzyme Activity

Preparation of enzyme extract:

The enzyme extract preparation was carried out according to the method of Applebaum *et al.,* (1964) with some modification. Only midgut was taken from both larvae and pupae, kept in ice-cold insect Ringer's solution, accurately weighed and homogenized for 3 minutes at 0°C in ice-cold citrate phosphate buffer (pH–6.8) using pestle and mortar. The midgut was suspended in ice–cold buffer and made up to 1 ml, the homogenate was centrifuged at 10,000 rpm for 15 minutes and the supernatant was used as an enzyme for estimation of digestive enzyme activity. The enzyme activity of amylase and invertase were estimated by the methods of Ishaaya and Swirsky (1970), while protease activity by Snell and Snell (1971) method and lipase activity by the method of Cherry and Crandel (1932).

Preparation of midgut for bioassay experiment:

Alimentary canal was dissected out in insect saline, the midgut was separated, contents of the midgut were removed by injecting the insect saline into the open midgut tube and contents were flushed out. The epithelial tissue was washed in insect saline and transferred to fresh saline. The extract of the midgut epithelia was prepared, the homogenate having a concentration equivalent to two midgut epithelia / 10 ml saline was bioassayed for its effect on in vitro digestive enzyme secretion in preparation of the midgut. The midgut preparation was incubated with 2 ml of incubation solution (midgut epithelial extract) in the bioassay apparatus for 30 minutes with a bubbling gentle stream of oxygen. After incubation, the midgut preparation was taken out and washed in insect saline, the gut was opened and contents were collected in 0.5 ml distilled water for estimation of protease and amylase activity. In control experiments, ligated midgut tubes were incubated in insect saline.

SDS-PAGE Electrophoresis

Midgut extraction was carried out by the method of Laemmli, (1970) with some minor modifications (Barsagade, 1998). The molecular weight of the protein bands was estimated with the help of Gel.Doc.

Results

Biochemical Analysis

Concentration of the biomolecules present in the midgut epithelial cells of larvae and pupae were found as-

Total DNA concentration

The total midgut DNA concentration in fifth instar was about 0.58 ± 0.08 µg/mg, it decreased to 0.3 ± 0.06µg/mg in early pupa. The total DNA concentration thereafter increased gradually up to 0.45 ± 0.04 µg/mg and 0.66 ± 0.08 µg/mg in mid pupa and late pupa respectively (Fig.1).

Total RNA concentration

The total RNA concentration in the fifth instar larvae was estimated as 12.2 ± 0.99 µg/mg while it was 7.12 ± 0.59 µg/mg in early pupa, increased to 9.12 ± 1.01 µg/mg in mid pupa and was found to be 18.6 ± 1.01 µg/mg in the late pupal stage (Fig-2).

Total Protein concentration

The total midgut protein concentration in fifth instar was about 4.5 ± 0.29 µg/mg. It decreased to 2.04 ± 0.22µg/mg in early pupa, the total protein concentration thereafter increased gradually up to 3.73 ± 0.31 µg/mg and 6.5 ± 0.14 µg/mg in mid pupa and late pupa respectively (Fig-3).

Total Carbohydrate concentration

The total carbohydrate concentration in the fifth instar larvae was estimated as 0.70 ± 0.0046 µg/mg while it was 0.67 ± 0.0023 µg/mg in early pupa, increased to 0.68 ± 0.0021 µg/mg in mid pupa and was found to be 0.72 ± 0.0041µg/mg in the late pupal stage (Fig-4).

Midgut Digestive Enzyme

The digestive enzymes, amylase, invertase, protease, and lipase in the midgut have been demonstrated qualitatively and quantitatively. Enzyme estimation in the larval and pupal stages is summarized in table 1. and fig.5.

Table-1: Enzyme estimation in larval and pupal stages of *Apis cerana indica*. (Mean ± SD)

S. No.	Enzyme	V-instar µg/mg	Early Pupa µg/mg	Mid Pupa µg/mg	Late Pupa µg/mg
1.	Amylase	0.19± 0.0054	0.53± 0.0187	0.028± 0.022	0.32± 0.014
2.	Invertase	0.34± 0.015	0.44± 0.0122	0.77± 0.015	0.80± 0.055
3.	Protease	0.75± 0.0057	0.78± 0.01	0.43± 0.0291	0.82± 0.021
4.	Lipase	4.5± 0.05	5.5± 0.291	2.9± 0.212	3.2± 0.254

Effect of Midgut Extract

Bioassay experiment was conducted to study the effect of midgut extract on the midgut amylase and protease activity in the fifth instar larvae of honey bee *Apis cerana indica* (Fig.6). During the bioassay experiment the midgut extract showed the significant ($P<0.0001$) effect elevating amylase and protease activity in fifth instar.

Amylase activity:

The midgut amylase activity was measured about 0.71 ± 0.089 mg glucose/midgut /minute after the incubation in midgut extract for 30 minutes while, it was observed 0.51 ± 0.071 mg glucose/midgut/minute in the normal condition.

Protease activity:

The midgut protease activity was measured about 0.94 ± 0.091 mg protein/midgut/minute after incubation in midgut extract for 30 minutes while, it was noticed about 0.79 ± 0.083 mg protein/midgut/minute in normal condition.

Electrophoretic Analysis

The SDS PAGE electrophoretic analysis of midgut extract demonstrated about eleven bands of protein among which six bands were predominant ranging from 26-65 kDa molecular weight in first to fifth instar larvae, early, mid and late pupae of *Apis cerana indica* .While an additional band of 12KD protein was found only in late pupa (Fig. 7).

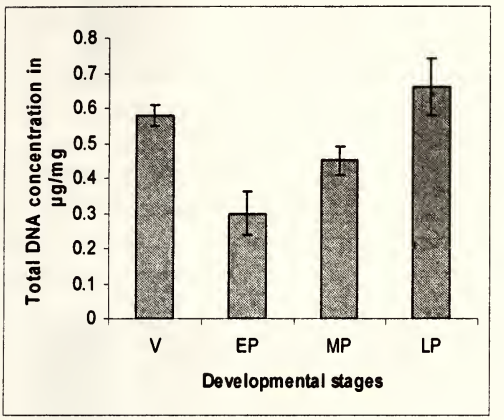

Fig. 1 Total DNA concentration during post-embryonic development

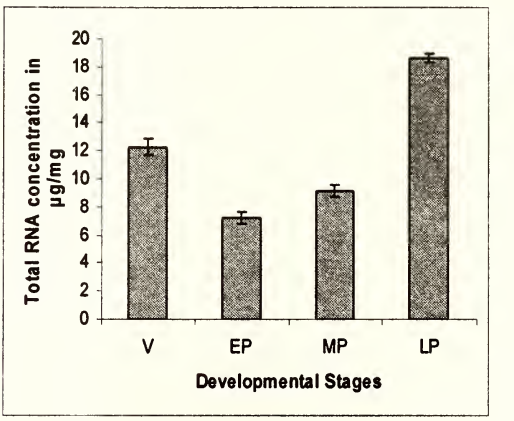

Fig. 2 Total RNA concentration during post-embryonic development.

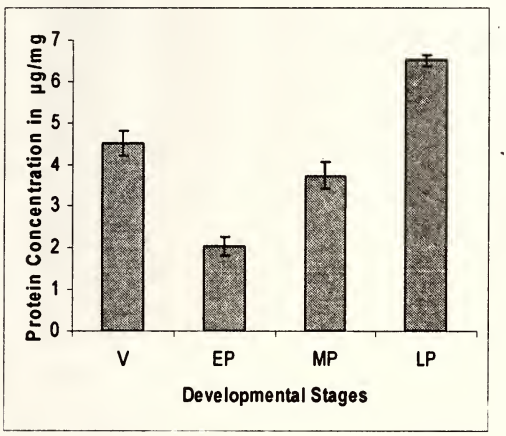

Fig. 3 Total protein concentration during post-embryonic development.

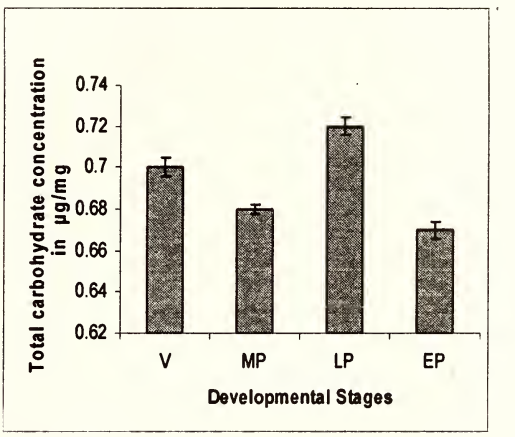

Fig. 4 Total carbohydrate concentration during post - embryonic development.

Abbreviations : V – Vth Larval stages ; EP- Early pupa ; MP – Mid pupa ; LP –Late pupa

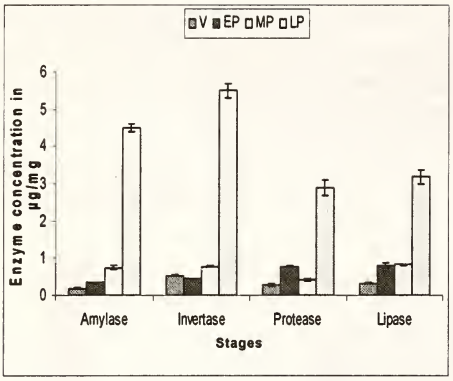

Fig. 5 Estimation of enzymes in larval and pupal stages during post-embryonic development.

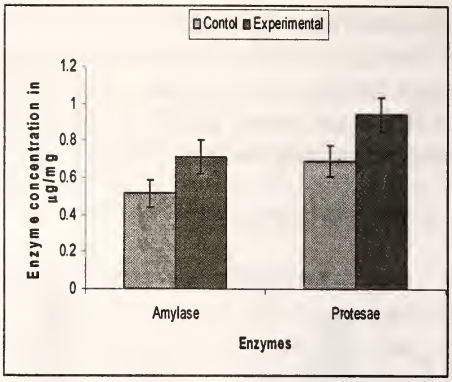

Fig. 6 Effect of midgut extract on midgut activity in V-instar larva.

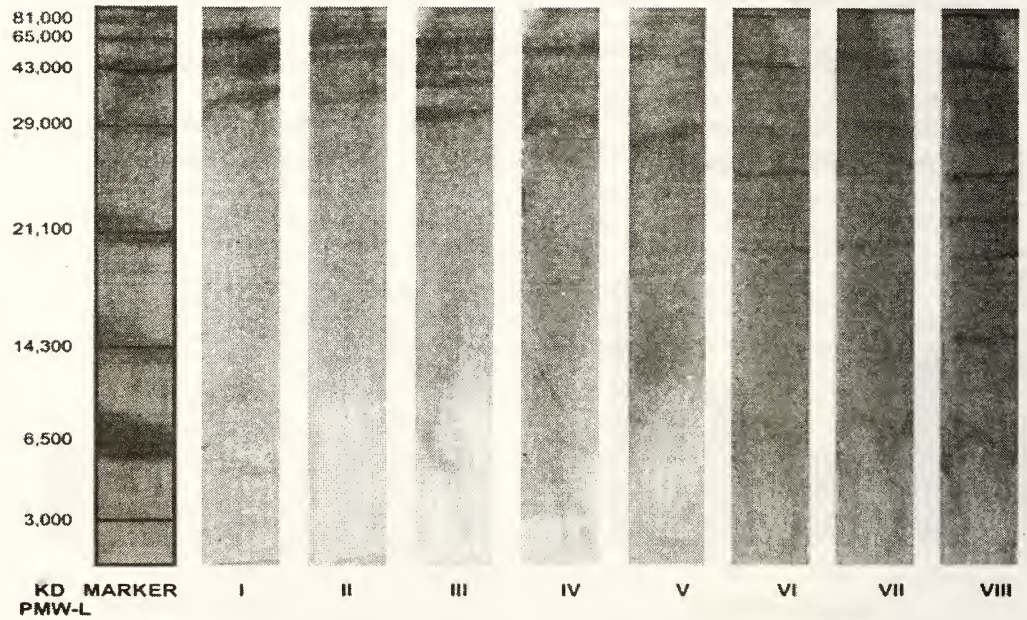

Fig. 7 : SDS-PAGE analysis of the midgut extract of larvae of *Apis cerana indica* showing the presence of protein pattern.

Abbreviation: I- first instar, II- second instar, III - third instar, IV - fourth instar and V- fifth instar, VI-early pupa, VII- mid pupa, VIII-late pupa.

Discussion

During the post embryonic development, the differentiation of midgut epithelial cells occurs in honey bee (Chapman, 1985a and 1985b and Cruz-landim and Cavalcante, 2003) while in the successive development, replacement of larval midgut epithelial cells to adult by formation of new epithelium is found in *Apis cerana indica* (Barsagade and Kelwadkar, 2008). In the last larval instar the midgut epithelial cells, produce various digestive enzymes that help for the food digestion in *Apis mellifera* (Cavalcante and Cruz-Landim, 2004). During larval development midgut cells are replaced by the other cells depending upon death of larval digestive cells, proliferation of regenerative cells occurs in order to constitute a new digestive epithelium in the adult, *Apis mellifera* (Geogorc and Bowen, 1997) and in *Apis cerana indica,* (Barsagade and Kelwadkar, 2008).

The midgut columnar epithelial cells containing the biomolecules such as DNA, RNA and Proteins are actively engaged in the protein synthesis in order to secure various digestive enzymes during the larval–pupal metamorphosis (Fuji, 1979, Wigglesworth, 1972 and Chapman, 1998).

In *Apis cerana indica,* total DNA, total RNA, total proteins and total carbohydrates were intensely demonstrated in the nuclei and perikarya of columnar epithelial cells, as found in *Apis florea* and *Apis mellifera,* described by earlier workers showing their role in protein synthesis (Pearse, 1968; Ban and Prezlec, 1974). The similar role of these biomolecules may be played in the protein synthesis during metamorphosis in *Apis cerana indica.*

Various biochemical tests reveal the presence of amylase, invertase, protease and lipase activity in the midgut of larvae of *Apis cerana indica* which are very specific in their activity (Horie, 1970, Banergee and Saxena, 1983, Wajiro *et al.,* 1984 and Zufelato *et al.,* 2004). The higher level of enzyme acid phasphatase activity in the larval peritrophic membrane in *Apis mellifera* is mostly used for digestion (Cavalcante and Cruz-landim, 2004). Similarly, activity of other fourteen enzymes was determined in the midgut of *Apis mellifera* and these enzymes were alfa-amylase, 4 alfa-glucosidase, 3 protease, 5 amino peptidase, lipase etc. (Banerjee and Sexena,1983). According to Mahmoud (2007), the high food metabolism level depends on the protein concentration in the midgut and haemolymph, it may reflect the digestion level with acid phasphatase and other digestive enzymes. The present investigation reveals that the concentration of biomolecules viz. total DNA, total RNA, total protein and total carbohydrates in the fifth instar larvae of *Apis cerana indica*, shows initial depletion and then a significant rise because of involvement of total protein consumption in the midgut for synthesis of digestive enzymes.

The study also supports the findings of earlier works, showing the amylase, invertase, proteases and lipase activity during the larval-pupal metamorphosis in *Apis cerana indica.* Cavalcante and Cruz-landim (2004), noticed the mass range of different proteins varying form 19 to 142 kDa, increased greatly from larvae to pupae and tends to decrease during pupation, until the phase of brown eye pupae and increased again in the pupae with pigmented body (pharate adult). In *Apis cerana indica,* 11 bands were observed, among which six bands were predominant ranging from 26–65 kDa, found in the midgut extract of developing larvae and pupae, supporting the earlier studies.

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